SUPPLEMENTARY MATERIAL

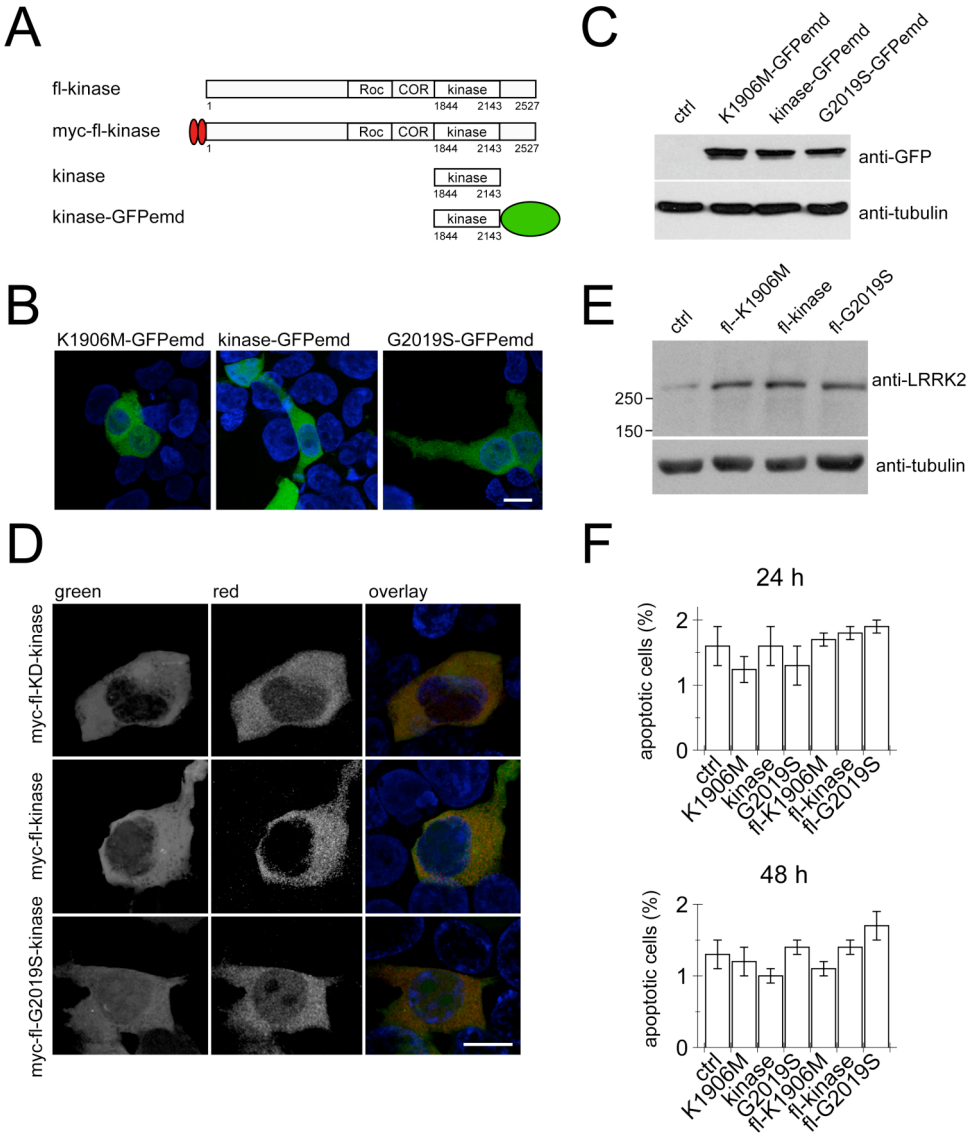

Figure S1 (related to Figure 1) Overexpression of LRRK2 constructs and effects on cell death. (**A**) Schematic representation of myc-tagged full-length LRRK2 (myc-fl-kinase) and non-tagged full-length LRRK2 (fl-kinase), as well as C-terminally tagged or non-tagged kinase domain (kinase) constructs employed, with domains and amino acids indicated. (**B**) Representative maximum-intensity projection of confocal images of cells transfected with GFP-tagged catalytically inactive K1906M-mutant kinase, wildtype kinase or G2019S-hyperactive kinase domain constructs as indicated, and processed 48

h after transfection. Scale bar, 10 μm. (**C**) As in (**B**), but cell extracts prepared and analyzed for levels of overexpression using a GFP antibody, and using a tubulin antibody to correct for differences in protein loading. (**D**) Representative maximum-intensity projection of confocal images of cells co-transfected with GFP (green) and myc-tagged full-length wildtype (myc-fl-kinase) or mutant LRRK2 constructs (G2019S or catalytically dead (KD)) as indicated, fixed 48 h after transfection and stained with an anti-myc antibody (red). Scale bar, 10 μm. (**E**) Cells were transfected with non-tagged, full-length wildtype or mutant LRRK2 constructs as indicated, and cell extracts prepared and analyzed with an anti-LRRK2 antibody (MJFF2, Epitomics) 48 h after transfection for levels of overexpression, and with a tubulin antibody to correct for differences in protein loading. (**F**) Cells were co-transfected with mCherry and with either empty vector (ctrl) or with the indicated kinase or full-length (fl) kinase constructs, and cell death analyzed either 24 h (top) or 48 h (bottom) after transfection using Hoechst staining. Bars represent mean ± s.e.m. (n=3).

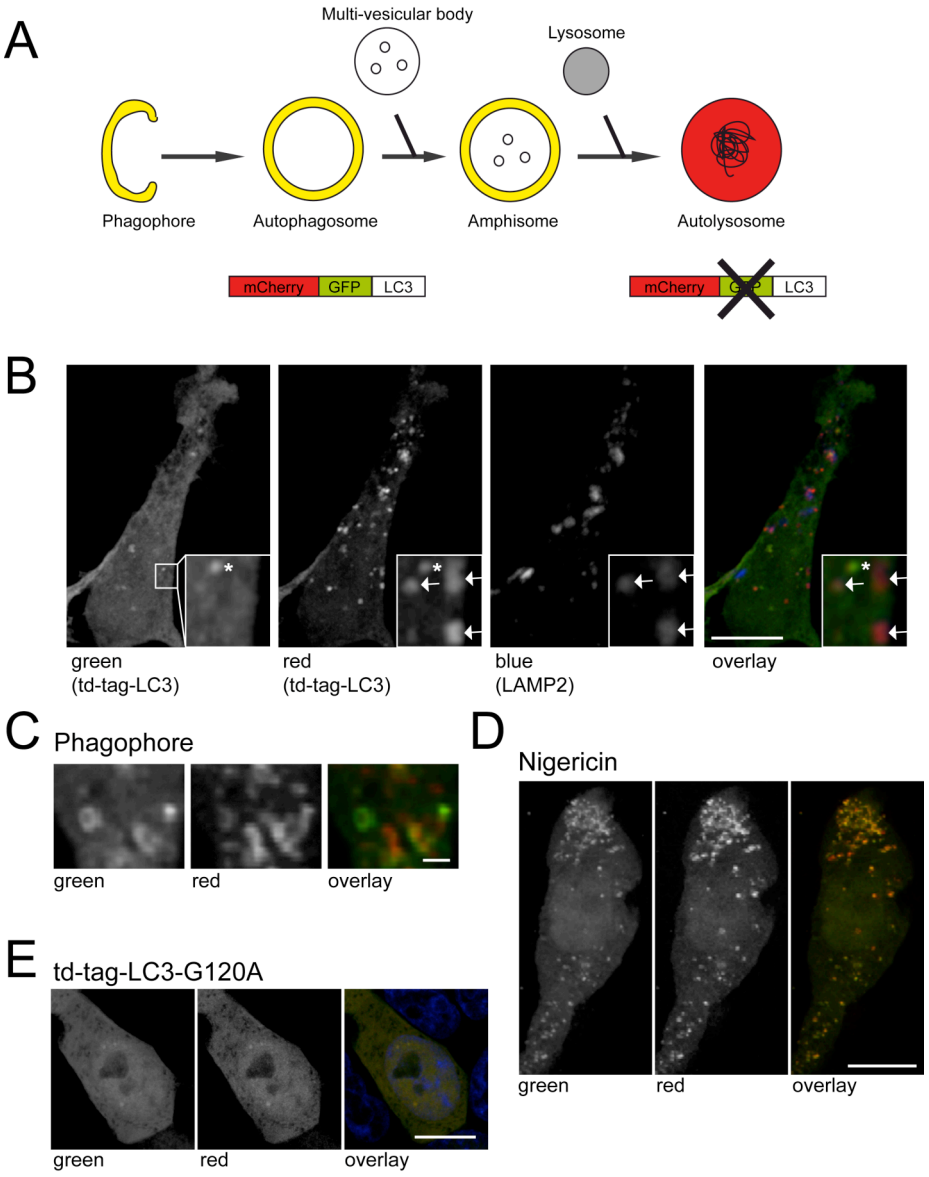

Figure S2 (related to Figure 2) Characterization of td-tag-LC3 labeling. (**A**) The tandem-tagged (td-tag) LC3 protein emits yellow (green merged with red) fluorescence in non-acidic structures, but appears as red only in autolysosomes due to the quenching of the GFP in the acidic environment. (**B**) Confocal image of a cell transfected with td-tag-LC3 and co-stained with LAMP2 (blue). Scale bar, 10 μm. Inset: Example of yellow, early autophagic structures negative for LAMP2 (asterisk) and red-only, late autophagic structures positive for LAMP2 (arrows). (**C**) Detection of a phagophore with td-tag-LC3. Scale bar, 1 μm. (**D**) Confocal image of a cell transfected with td-tag-LC3

and treated with nigericin (25 μM, 5 min), a H^+/K^+ ionophore which increases lysosomal pH, thus allowing visualization of both early and late autophagic structures as yellow punctae. Scale bar, 10 μm. (**E**) Confocal image of a cell transfected with G120A-mutant td-tag-LC3, which cannot be processed and conjugated to the autophagosomal membrane, and thus appears only as cytosolic fluorescence. Scale bar, 10 μm.

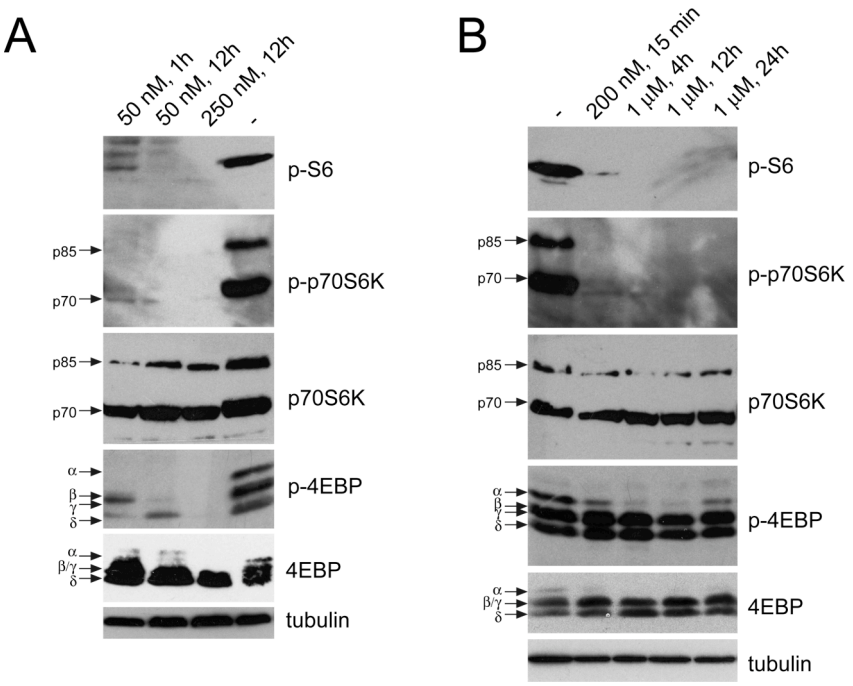

Figure S3 (related to Figure 3) Positive controls for regulation of TORC1 signaling. (**A**) Example of an experiment where cells were either left untreated (-), or treated for the indicated times with the indicated concentrations of torin, followed by analysis of cell extracts for the various substrates related to TORC1 activity. (**B**), as in (**A**), but cells treated with rapamycin as indicated, and cell extracts analyzed for the indicated TORC1 substrates. As previously described, both rapamycin and torin block TORC1-dependent phosphorylation of p70S6K, which in turn blocks phosphorylation of S6K. In contrast, only torin is able to suppress the TORC1-dependent but rapamycin-resistant phosphorylation of 4E-BP1. Experiments of this type were performed two times with similar results.

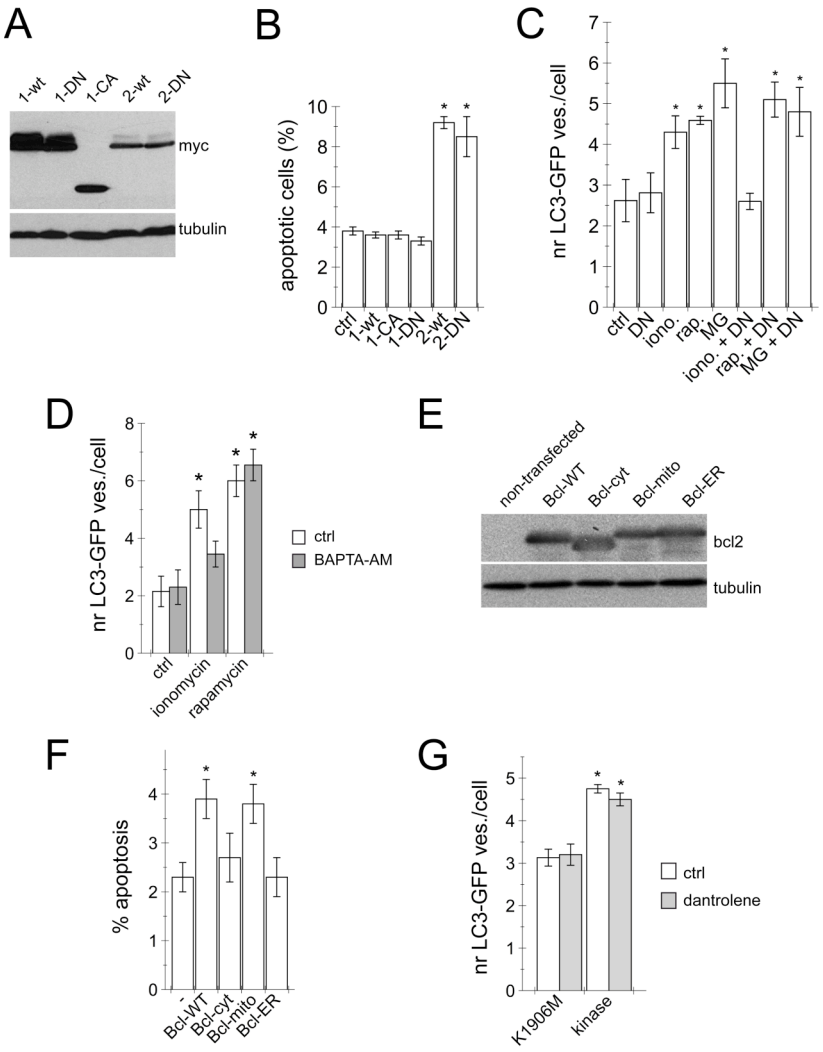

Figure S4 (related to Figure 4) Effects of LRRK2 overexpression on AMPK and intracellular calcium. (**A**) Cells were transfected with N-terminally myc-tagged human wildtype (1-wt), dominant-negative (1-DN) or constitutively active (1-CA) AMPKα1, or with wildtype (2-wt) or dominant-negative (2-DN) AMPKα2, and cell extracts analysed for expression with an anti-myc antibody 48 h after transfection. (**B**) Cells were transfected with the indicated AMPK constructs, and analyzed for cell death using Hoechst staining 48 h after transfection. Bars represent mean ± s.e.m. (n=3); *, $p < 0.005$. (**C**) Cells were co-transfected with LC3-GFP and either empty vector or dominant-negative AMPKα1 (DN) as indicated, and treated with ionomycin (2.5 μM,

12 h) (iono.), rapamycin (100 nM, 12 h) (rap.) or MG-132 (500 nM, 12 h) (MG) as indicated, and LC3-GFP punctae quantified 48 h after transfection. Bars represent mean ± s.e.m. (n=3); *, $p < 0.05$. (**D**) Cells were transfected with LC3-GFP and either left untreated (ctrl) or treated with ionomycin (2.5 μM, 12 h) or rapamycin (100 nM, 12 h) as indicated, and either left untreated (white bars) or treated with BAPTA-AM (5 μM, 2 h) (grey bars) prior to analysis for LC3-GFP punctae 48 h after transfection. Bars represent mean ± s.e.m. (n=3); *, $p < 0.05$. (**E**) Cells were transfected with the indicated bcl chimerae, and extracts analyzed for overexpression levels 24 h after transfection with an anti-bcl antibody. (**F**) Cells were transfected with either empty vector (-) or the indicated Bcl chimerae, and analyzed for cell death using Hoechst staining 24 h after transfection. Bars represent mean ± s.e.m. (n=3); *, $p < 0.05$. (**G**) Cells were co-transfected with LC3-GFP and either K1906M or kinase domain constructs, and either left untreated (white bars) or treated with dantrolene (1 μM, 2 h) (grey bars) prior to analysis for LC3-GFP punctae 48 h after transfection. Bars represent mean ± s.e.m. (n=3); *, $p < 0.05$.

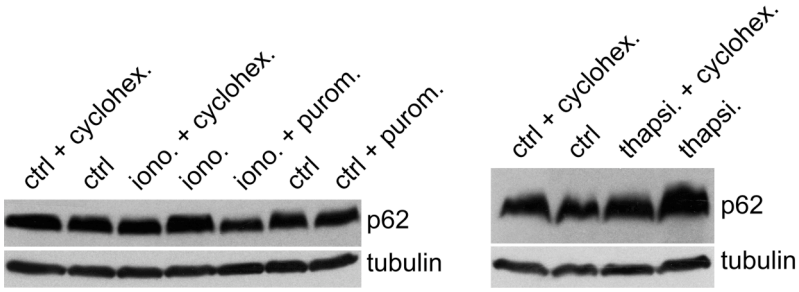

Figure S5 (related to Figure 5) Effects of intracellular calcium on p62 levels. Representative experiment where cells were either left untreated (ctrl), or incubated with ionomycin (2.5 μM, 6h) or thapsigargin (100 nM, 6h) to increase cytosolic calcium levels. Where indicated, cells were treated with puromycin (1 μM, 6h) or cycloheximide (350 nM, 6h) before analysis of p62 levels by Western blotting. The experiment was repeated once with similar results.

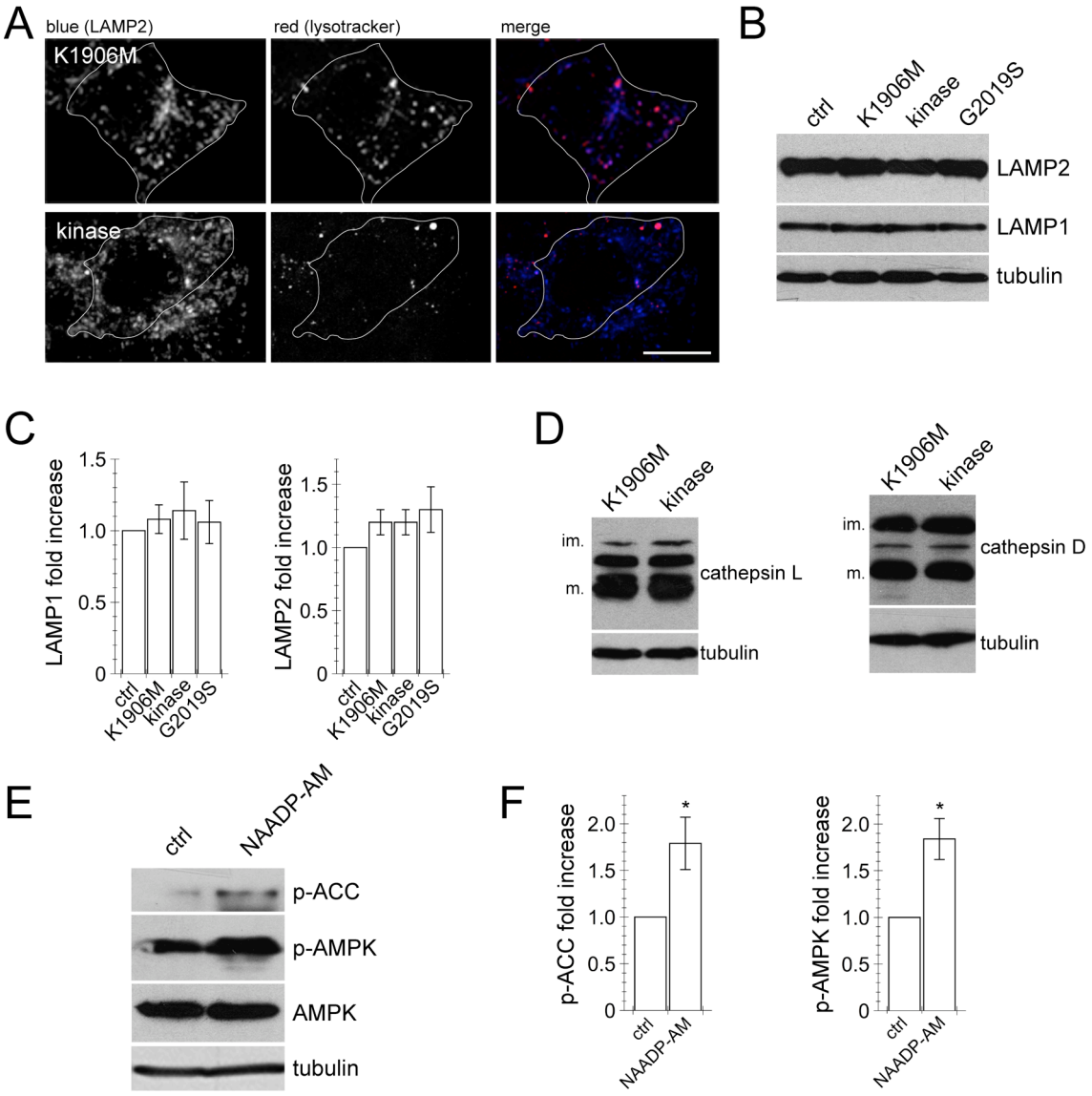

Figure S6 (related to Figure 6) Effects of LRRK2 on lipid accumulation and lysosomal pH, and effects of NAADP-AM. (**A**) Example of cells co-transfected with EGFP and either K1906M or kinase domain constructs, and stained with anti-LAMP2 (blue) and lysotracker (red). Scale bar, 10 μm. (**B**) Cells were transfected with empty vector (ctrl) or the indicated constructs, and extracts analyzed for levels of LAMP1 and LAMP2 by Western blotting. (**C**) Quantification of experiments as in B, where bars represent mean ± s.e.m. (n=3). (**D**) Cells were transfected with the indicated constructs and analyzed for the processing of cathepsin L (left) or cathepsin D (right). (**E**) Cells

were treated with NAADP-AM (100 nM, 12 h) before analysis for levels of p-ACC and p-AMPK as indicated. (**F**) Quantification of experiments as in E, where bars represent mean ± s.e.m. (n=3); *, $p < 0.05$.

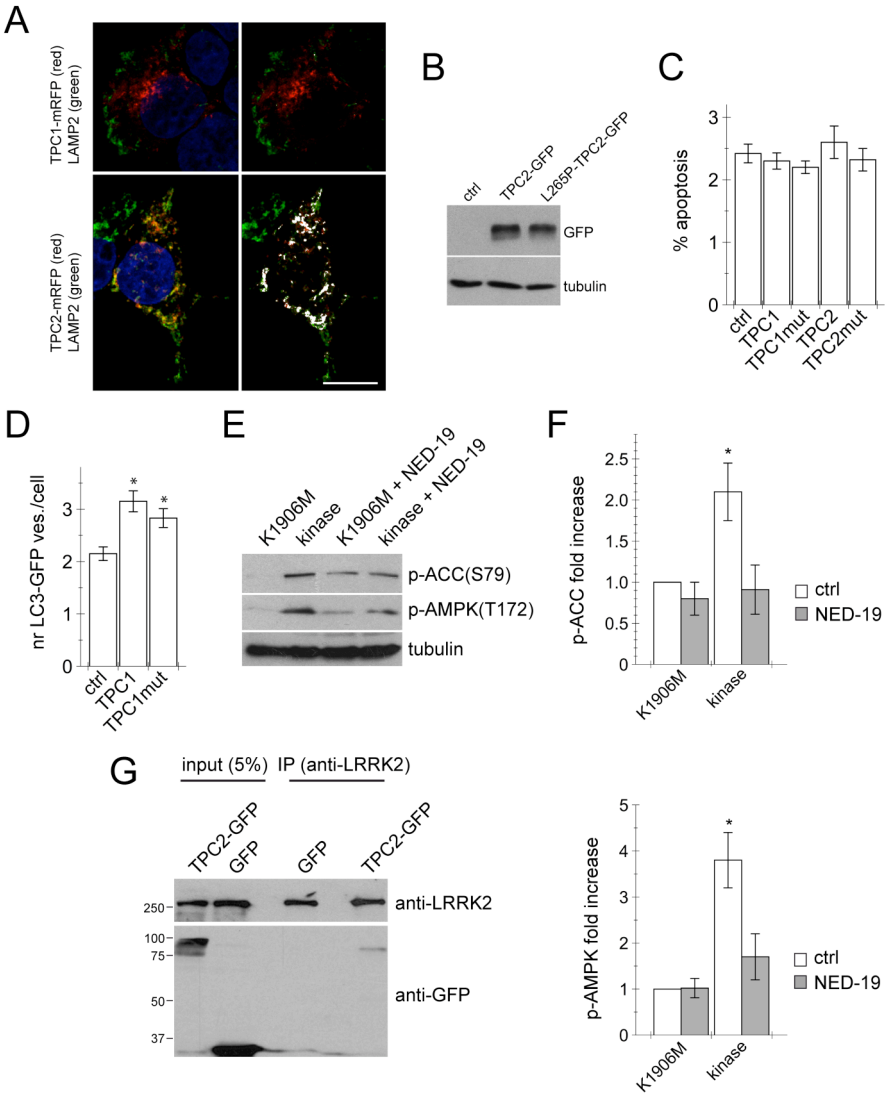

Figure S7 (related to Figure 7) A link between LRRK2 and NAADP-sensitive receptors. (**A**) Cells were transfected with the indicated RFP-tagged TPC constructs and stained for LAMP2 (green) and DAPI (blue). White: colocalization mask. Scale bar, 10 μm. (**B**) Cells were transfected with empty vector (ctrl) or the indicated GFP-tagged TPC2 constructs, and extracts analyzed for differences in overexpression using an anti-GFP antibody. (**C**) Cells were transfected with either empty vector (ctrl) or the indicated constructs, and analyzed for cell death using Hoechst staining 24 h after transfection. Bars represent mean ± s.e.m. (n=3). (**D**) Cells were co-transfected with LC3-GFP and

either empty vector (ctrl) or wildtype or mutant TPC1, and LC3-GFP punctae analyzed 24 h after transfection. Bars represent mean ± s.e.m. (n=4); *, $p < 0.05$. (**E**) Cells were transfected with either K1906M or wildtype LRRK2 kinase domain constructs, and treated with NED-19 (1 μM, 12 h) where indicated before analysis for p-ACC and p-AMPK. (**F**) Quantification of experiments as in E, where bars represent mean ± s.e.m. (n=3); *, $p < 0.05$. (**G**) Cells were transfected with TPC2-GFP or GFP vector, and extracts (1 mg) subjected to immunoprecipitation of endogenous LRRK2 with an anti-LRRK2 antibody (N138/6, NeuroMab, UCDavis, USA). Co-immunoprecipitated TPC2-GFP was detected using an anti-GFP antibody, and inputs (5%) were run along-side the immunoprecipitates. Representative of a total of four independent experiments. Note that TPC2-GFP runs as distinct bands corresponding to core glycosylated and mature fully glycosylated versions, respectively (67).

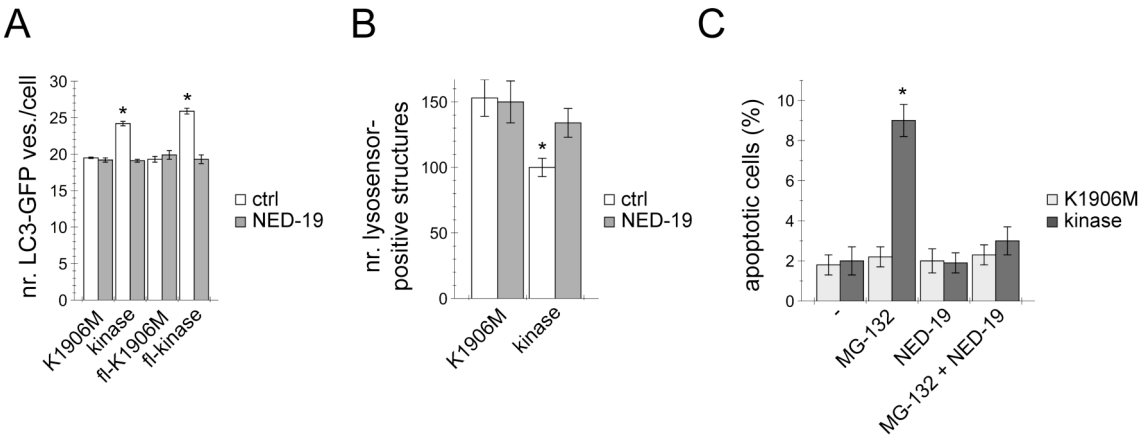

Figure S8 (related to Figure 8) Effects of LRRK2 expression on autophagy induction, lysosomal pH and cellular viability in the presence of proteasomal inhibitors in dopaminergic neuroendocrine PC12 cells. (**A**) Neuroendocrine PC12 cells were co-transfected with LC3-GFP and the indicated kinase domain or full-length LRRK2 (fl-kinase) constructs, and either left untreated (white bars) or treated with NED-19 (1 μM, 12 h) (grey bars), followed by quantification of LC3-GFP punctae 48 h after transfection. Bars represent mean ± s.e.m. (n=3); *, $p < 0.005$. (**B**) Transfected PC12 cells were treated with NED-19 (1 μM, 12 h) where indicated before staining with lysotracker. Bars represent mean ± s.e.m. (n=3); *, $p < 0.05$. (**C**) PC12 ells were co-transfected with GFP and K1906M or kinase domain constructs as indicated and either left untreated (-), treated with MG-132 (500 nM, 12 h), NED-19 (1 μM, 12 h) or both as indicated. Cells were analysed for cell death 48 h after transfection using Hoechst staining. Bars represent mean ± s.e.m. (n=3); *, $p < 0.005$.